Allergic Fungal Rhinitis and Rhinosinusitis

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The intent of this article is to review the published literature addressing the role of fungi as causative agents in allergic rhinitis and rhinosinusitis. Ambient mold spores are widely distributed in nature, and an estimated 3 to 10% of the world's population is allergic to molds. There are compelling epidemiologic links between mold (fungal) allergy and illnesses such as asthma and "asthma with allergic rhinitis." Fungal allergy is more prevalent in areas of high ambient mold spore concentrations. However, epidemiologic studies have failed to demonstrate a direct relationship between fungal allergy and allergic rhinitis either via outdoor or indoor exposure. Fungal allergy is clearly linked to a subset of chronic rhinosinusitis (CRS) known as allergic fungal rhinosinusitis (AFRS). This condition represents an intense allergic response against colonizing fungi giving rise to formation of allergic (eosinophilic) mucin, mucostasis, and sinus opacification. A broader role for colonizing fungi has been postulated in CRS owing to the demonstration of fungi in mucus in the vast majority of cases of CRS, and *in vitro* studies demonstrating that certain fungi, particularly *Alternaria*, elicit a "modified" allergic response in patients with CRS that is independent of IgE.

Keywords: allergic rhinitis; rhinosinusitis; fungal; allergy; mold

Fungal spores are broadly distributed in nature, but demonstrate distinct regional trends owing to latitude, climatic differences, humidity, and probably other factors such as vegetation. In indoor environments, the substrate for fungal growth is another important variable (1). Spore counts typically exceed pollen counts by 100-fold. Fungi are distinct from other allergen classes due to the fact that they are viable and can germinate in nasal and sinus mucus as well as indoor environments. Fungi have the capacity to cause infections, allergic or hypersensitivity responses, or toxic-irritant effects (1). The latter is due to production of mycotoxins, which have known toxic effects but health effects that are difficult to assess. Given these facts, it is perhaps not surprising that fungal spores would be associated with the full spectrum of respiratory allergic disease, from allergic rhinitis (AR) to allergic rhinosinusitis and allergic asthma. However, despite these facts, much less effort has gone into tracking and reporting on the density of fungal spores in outdoor air samples from around the world, and this has been one obstacle in establishing a causal relationship between fungi and diseases such as allergic rhinitis.

The intent of this article is to review the published literature addressing the role of fungi as causative agents in allergic rhinitis and rhinosinusitis. I shall take the prerogative of using the term "rhinosinusitis" rather than "sinusitis," although these terms should be viewed as interchangeable (2). Chronic rhinosinusitis (CRS) will be defined based on a duration of $\geqslant$ 12 weeks, typical symptoms (at least 2) of nasal congestion, facial pain/pressure or fullness, anterior or posterior nasal drainage and loss of sense of smell with objective evidence of sinus disease by endoscopic examination or radiographic imaging (3). I also use the terms "fungal" and "mold" interchangeably with no disrespect intended toward taxonomists, botanists, or mycologists. This is because the literature as it pertains to allergy has tended to use these terms loosely and interchangeably.

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ALLERGIC RHINITIS

Prevalence of Outdoor Fungal Allergy in Different Geographic Regions

Fungi are ubiquitous in outdoor ambient samples. Horner and coworkers estimated that between 3 and 10% of adults and children worldwide are allergic to molds (4). However, studies of the prevalence of sensitization by allergen class from around the world generally place fungal allergy below sensitization to other allergen classes, even in tropical areas where spore densities are highest. This is true in tropical climates (such as Malaysia, Puerto Rico, and Singapore), temperate climates (Midwestern United States), and in arid or semi-arid climates (Finland; Kuwait; and Tucson, Arizona). In tropical and other hot, humid regions, spore counts typically exceed pollen counts by 100-fold. Yet, in these studies, AR has generally been more strongly associated with outdoor pollen sensitization than indoor allergen sensitization. Only the study by Kidon and colleagues (5) showed an unusually low prevalence of pollen allergy in a pediatric population in Singapore. For instance, a study of allergic children in Tucson, Arizona found that sensitization to Bermuda grass was most prevalent among children with AR or control subjects, whereas sensitization to *Alternaria* was most prevalent in children with asthma (6). In fact, *Alternaria* was the only allergen independently associated with increased risk for asthma both at age 6 and at age 11. Boulet and coworkers (7) studied 3,371 consecutive patients for prevalence of allergies and relation to clinical condition. Among 195 patients only sensitized to outdoor allergens (pollens), 73.8% had AR, 11.8% asthma, and 14.4% had both. Among 710 patients sensitized only to indoor allergens (house dust, cat, dog, or mite), 48.6% had AR, 24.5% had asthma, and 26.9% had both. Among 1,793 patients sensitized to both indoor and outdoor allergens, 55.5% had AR, 14.6% had asthma, and 29.9% had both. Among those sensitized to *Alternaria* (considered outdoor and indoor allergen), 25.8% had AR alone, 20.9% had asthma, and 27.4% had both. Similarly, Ezeamuzie and colleagues (8) examined the prevalence of allergen sensitization in 810 patients in Kuwait with either asthma or AR. They found that the prevalence of allergy to at least one mold was 20.9%, whereas the prevalence in 120 matched control subjects was 5.8%. The prevalence was much higher, however, among patients with asthma alone (45. 8%) or both asthma and rhinitis (28.3%) than those with rhinitis alone (11.8%) ($P < 0.001$).

Another way to address this question is to ask whether mold-induced allergy is more prevalent in environments of high ambient spore counts. Based on the studies summarized in Table 1, the answer appears to be yes. The study from Finland (9) found a prevalence of sensitization to *Alternaria alternata* and *Cladosporium herbarum* of only 2.8% and 2.7%, respectively, whereas studies of these and other fungi in more temperate or tropical areas placed the prevalence of allergy

TABLE 1. PREVALENCE OF ALLERGY BY ALLERGEN CATEGORY IN PATIENTS WITH ALLERGIC RHINITIS BASED ON OUTDOOR STUDIES

	Prevalence of Allergy by Allergen Class					
Location of Study	Pollen Allergy	Dust Mite Allergy	Cockroach Allergy	Animal Dander Allergy	Fungal (Mold) Allergy	Reference
Israel	43% (tree) 53% (grass) 40% (weed)	84%	34%	40–53%	30%	(78, 79)
Puerto Rico	31.1%	94.3%	41.5%	31.5%,	19.4%	(79)
Ankara, Turkey	–	–	–	–	14.8%	(80)
Singapore	15%	97%	–	20%	19%*	(5)
Malaysia, Kuantan	–	–	–	–	Fusarium 23.5% Aspergillus 21.2%†	(81)
Finland	–	–	–	–	2.8% 2.7%	(9)
Kuwait	54.6%	41.2%	–	–	20.9%	(82)
Italy	–	–	–	–	10.4% total	(83)
Chicago, Illinois	–	–	–	–	44%	(56)
California	10–42% trees 46–54% grasses 19–37% weeds	53%	23%	39% (cat)	11–22%	(84, 85)

* The prevalence of fungal allergy was 49% in patients living in homes without air conditioning and 10% in patients living in homes with air conditioning.
† The prevalence for other fungi was: 18.8% *Dreselera orysae*, 17.6% *Alternaria* sp., 17.6% *Curvularis eragrostidis*, 16.5% *Penicillium oxa*, 16.5% *Pestolotriopsis gtuepini*, 16.5% *Rhizopphus arrhi*, 15.3% *Aspergilluls nigus*, 12.9% *Penicillium choy*, 11.8% *Aspergillus fumigatus*, and 4.7% *Cladosporium* sp.

around 20%. However, these studies were not designed to establish causation between mold allergy and allergic rhinitis.

A study by Andersson and coworkers (10) attempted to address the issue of causation between mold allergy and allergic rhinitis in a study of children in inland New South Wales, Australia. They found a correlation between allergic rhinitis symptoms and airborne *Alternaria* spore concentrations that remained even after excluding children with ryegrass allergy. Gergen and Turkeltaub examined the association of individual allergen reactivity with respiratory disease using the NHANES database (11). In this study of the white civilian U.S. population (n = 4,295) aged 6 to 24 years, the independent association of individual allergen reactivity with asthma and allergic rhinitis was assessed using logistic models. Asthma was associated with reactivity to house dust and *Alternaria*. These associations were also seen in the asthma-only (without allergic rhinitis) group. Allergic rhinitis was associated with reactivity to ragweed, ryegrass, house dust, and *Alternaria*. However, in the allergic rhinitis only (without asthma) group, the association of allergic rhinitis with *Alternaria* was not seen.

While the studies summarized do not conclusively establish molds as a cause of allergic rhinitis, they do indicate that a considerable fraction of patients with allergic rhinitis (with or without associated asthma) have mold allergies. Part of the problem of attributing allergic rhinitis symptoms to outdoor environmental spore counts is the difficulty defining a clear-cut window of spore exposure independent of outdoor pollen allergies.

Are Indoor Molds a Relevant Cause of Allergic Rhinitis?

There is also a paucity of data clearly linking allergic rhinitis to indoor mold exposures. A major limitation of the data is the lack of quantitative mold sampling and potential confounding of rhinitis symptoms with infection (1). Many studies have relied on self-reports of indoor dampness or visible mold in homes. A study by Koskinen and colleagues (12) in Finland found an association between self-reported moisture in the home and rhinitis symptoms (odds ratio [OR], 1.89; confidence interval, 1.15–3.11). A second study found a similar relationship in children from the same homes (13). Kilpeläinen and coworkers surveyed Finnish first-year university students and found an association between visible mold or "visible mold or damp stains or water damage" and allergic rhinitis (14). A study by Brunekreef and colleagues found a relationship between hay fever and "mold" or "dampness" in homes in a study of children in Canada (ORs, 1.57 and 1.26, respectively) (15). In another study, Su and coworkers found a correlation of childhood hay fever symptoms and indoor concentrations of *Cladosporium* spp., *Epicoccum* spp. Yeasts, and *Aureobasidium* spp. in a study of 150 homes in Topeka, Kansas (16).

In most cases, indoor mold prevalence is governed primarily by outdoor sources (17). This is true primarily for the above-ground portion of homes and buildings, but is not true of basements. Certain molds have been more closely associated with indoor dampness or mold growth in buildings. These include: *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus penicilloides*, *Exophiala* spp., *Phialophora* spp., *Stachybotrys chartarum*, *Trichoderma* spp., *Ulocladium* spp., and *Wallemia* spp. (18).

Do We See Epidemics of Allergic Rhinitis at Times of High Spore Counts (e.g., Flooding)?

The devastating natural disaster of Hurricane Katrina resulted in the flooding of countless homes. One study by the Centers for Disease Control (CDC) in the aftermath of flooding in New Orleans and surrounding parishes found that 46% of the inspected homes had visible mold growth (19). The study by Solomon and colleagues found that the outdoor spore counts in flooded areas were roughly double those in nonflooded areas (66,167 versus 33,179 spores/m^3; $P < 0.05$) with the highest spore concentrations being found in homes (up to 645,000 spores/m^3) (20). The most common mold species found were *Cladosporium*, *Aspergillus*, and *Penicillium*.

Under conditions of high levels of indoor exposure (fungal concentrations > 90th percentile), an association was also found between in-home fungal concentrations and development of allergic rhinitis in the first 5 years of life in a prospective birth cohort of 405 children of asthmatic/allergic parents from metropolitan Boston (21).

Results of Allergen Challenge Studies with Fungal Extracts

Several studies have shown that fungal allergens can elicit positive responses in nasal allergen challenges (22, 23). Helbling and coworkers (24) showed that the extracts of Basidiospores

could induce immediate nasal allergic responses in sensitized patients.

What about Local IgE-mediated Allergy in AR?

Unfortunately, there have been very few studies addressing this point. In one recent study, Wierzbicki and colleagues (25) performed nasal allergen challenges in 20 patients with non-allergic rhinitis. Each subject underwent a nasal challenge with glycerin, *Alternaria*, cockroach, timothy grass, cat hair, and *Dermatophagoides pteronyssinus*. Five patients developed positive challenges to seven allergens, but on repeated challenges with the same allergens all five patients had negative responses. None of the patients had a positive challenge with *Alternaria*. This study concluded that there was no evidence for "local allergy" in patients with perennial rhinitis and negative skin prick tests.

ALLERGIC FUNGAL RHINOSINUSITIS

Allergic fungal rhinosinusitis (AFRS) is a distinct syndrome accounting for between 5 and 10% of chronic rhinosinusitis (CRS) cases.

History of AFRS

AFRS was first recognized by Safirstein as an upper airway manifestation of allergic bronchopulmonary aspergillosis (ABPA) (26). The name "allergic aspergillosis sinusitis" was first proposed by Katzenstein and colleagues in Chicago (27). Subsequent case series found that *Aspergillus* was a less common cause of AFRS than other dematiaceous fungi. These reports came primarily from the southwest and southeast portions of the United States. For instance, a study by Manning and coworkers of 22 cases of AFRS found that dematiaceous fungi were found in 18 cases, whereas *Aspergillus* was found in only 1 case (28). The geographic differences in fungal representation in AFRS was confirmed by Ferguson and colleagues (29).

AFRS is a strictly noninvasive localized hypersensitivity response to fungal growth that arises in areas of compromised mucus drainage (1, 30). In contrast, acute and chronic invasive forms of fungal sinusitis occur mainly in diabetics or immunocompromised hosts (31–33). These conditions must be excluded by pathologic analysis in cases of AFRS. A fungal ball represents a noninvasive sludging of mucus and fungus without an immunologic response (34).

Diagnostic Criteria for AFRS

Diagnostic criteria have been outlined by deShazo (34) and others and were reviewed in a recent consensus conference (35). The criteria include: (*1*) the presence of chronic rhinosinusitis (nearly always in association with nasal polyposis); (*2*) the presence of "allergic mucin" (also referred to as "eosinophilic mucin") containing noninvasive fungal hyphae in one or more sinus cavities; (*3*) immunocompetence; and (*4*) fungal allergy. Certain radiographic features of disease are highly characteristic or "pathognomonic," but not essential for the diagnosis. In most cases the diagnosis is established after sinus tissue and mucus have been surgically removed and reviewed pathologically with fungal stains or fungal cultures. At the time of surgery, the patient might have a persistently opacified sinus cavity, and eosinophilic mucus plus polypoid tissue might be found to account for this opacification. Patients nearly always have type I allergic sensitivity to fungal antigens. Because of these distinctive features plus the distinctive complications of this disease, including bony erosion and facial dysmorphia, AFRS represents a distinct subset from the much broader group of patients with CRS.

Allergic fungal rhinosinusitis (AFRS) refers to CRS that is accompanied by sinus opacification with "allergic mucin," or thick, inspissated mucus that ranges in color from light tan to brown to dark green and contains degranulating eosinophils. Allergic mucin is generally identified at the time of surgery. Fungal hyphae are demonstrable within the allergic mucin, which is consistent with fungal colonization rather than invasive fungal disease. In contrast, in invasive fungal sinusitis the fungal hyphae penetrate the underlying mucosa. The patient must be confirmed allergic to the fungus present in the mucus. In practice, the fungal cultures may fail to confirm the fungus in the allergic mucin, therefore not allowing this final AFRS criterion to be strictly fulfilled. Nonetheless, most patients with AFRS are allergic to multiple fungi, including one or more commonly implicated in AFRS.

Clinical Presentation

The clinical presentation of AFRS may be similar to other cases of CRS with nasal polyps. AFRS is distinguished from CRS with NP by the presence of allergic mucin containing viable fungal hyphae (as demonstrated by fungal staining or culture) and evidence of IgE-mediated allergy to one or more fungi. Patients may have typical symptoms related to nasal polyps combined with production of semisolid mucus. Less commonly, the presentation may be dramatic, with acute visual loss or gross facial disfigurement with orbital abnormalities (28, 36–38)

Radiographic Imaging of AFRS

Most patients with AFRS have sinonasal polyposis, and therefore the imaging appearance may be indistinguishable from that condition. Both mucus accumulation and mucosal thickening contribute significantly to sinus opacification and are difficult to differentiate with sinus CT imaging. The maxillary, ethmoid, and sphenoid sinuses are most commonly involved. Unilateral involvement occurs in some cases.

The sinus CT findings in AFRS include foci of increased density within the opacified sinuses that corresponds to allergic mucin. Allergic mucin has areas of high protein content and low water concentration that give rise to characteristic "hyperdense" appearance on sinus CT and corresponding "hypointense" appearance on T2-weighted MR images (39). This is considered a pathognomonic feature of AFRS; however, it is more pathognomic for "allergic mucin" than for AFRS *per se*.

The presence of diffuse increased attenuation ("hyperdensities") within the paranasal sinuses and nasal cavity most likely represents AFRS or chronic hyperplastic rhinosinusitis and polyposis associated with desiccated retained mucosal secretions (concretions) (39). The decreased signal intensity on Tl- and very decreased signal intensity on T2-weighted MR images were hypothesized by Zinreich and colleagues (40) to be due to calcium as well as iron and manganese within the mucin. In a study of allergic mucin from two patients with proven AFRS, iron and manganese, both electromagnetic elements, were present in larger quantities compared with mucus in four patients with bacterial rhinosinusitis. It is now, however, known that the presence of inspissated mucosal secretions within the sinus cavity or along the crevices of polyps result in a markedly hypointense T2-weighted signal (39, 41). As a result, hyperdensities on sinus CT scan are most consistent with the presence of allergic mucin and suggestive but not pathognomonic of AFRS (42, 43).

Occasionally, sinus cavity opacification in AFRS may be associated with local pressure effects on bone. Bony demineralization of the sinus wall may ensue, resulting in expansion of the sinus and possibly mucocele formation. True bone erosion is

less common, occurring in 20% of cases in one series (44). The nasal cavity may also appear expanded.

Fungi Implicated in AFRS

The original description of "allergic aspergillus sinusitis" implicated *Aspergillus* as the cause and likened the disease process to (ABPA) (27). As previously mentioned, most subsequent case series of AFRS coming from the southwestern and southeastern United States attributed AFRS to dematiaceous fungi ("phaeohyphomycosis"), including *Bipolaris*, *Curvularia*, *Exserohilum*, and *Alternaria* spp. (45). *Aspergillus fumigatus* and other *Aspergillus* species ("hyalohyphomycosis") are causative in some cases (45, 46). *Epicoccum nigrum* and *Fusarium solani* have been isolated in other cases of AFRS (47).

Isolation of Fungi by Standard Laboratory Methods

Culturing of fungi from sinus mucus has traditionally been problematic, although the reasons for this are not entirely obvious. Ponikau and coworkers (48) described a protocol for collection of mucus from sinus surgical cases and specimen handling designed to increase the success rate of identifying fungi by staining. They collected mucus with a power microdebrider to avoid use of suction, manually removed mucus with inflamed tissue *en bloc*, placed the mucus on saline-moistened nonabsorbable paper to prevent dessication of the sample, fixed the mucus and tissue *en bloc* in formalin, and embedded the sample in parafilm. They recommended collecting multiple samples to increase yield, because "fungi are frequently scattered." For culturing fungus, Lebowitz and colleagues (49) emphasized treatment of specimens with Sputolysin (a phenolic benzylamine mucolytic agent) (Boehringer Ingelheim GmbH, Ingelheim, Germany) and chloramphenicol, plating on Sabouraud, ChromAgar/Candida, Mycosel, and Niger seed agar plates; and incubating at 30°C (or 37°C) for up to 1 month.

In practice, the yield of fungal cultures is variable (reportedly 64–100%, but lower in the author's experience), and many cases are confirmed based on the presence of allergic mucin with a positive fungal stain (3). Unlike a positive fungal culture, the histologic appearance of allergic mucin is critical to the diagnosis of AFRS.

Diagnostic Testing in AFRS

By definition, patients with AFRS should be allergic to one or more fungi; this would include allergy to the fungus colonizing their allergic mucin. In practice, however, fungal cultures of allergic mucin may be negative despite unequivocal evidence of fungal hyphae in the mucin. This may relate to technical difficulties in submitting or performing the fungal culture. Fungi are difficult to identify with certainty on fungal stains. Results of skin testing (using both prick and intradermal methods) and *in vitro* (e.g., ImmunoCAP) testing methods typically give comparable results, and most patients are allergic to multiple fungi. In establishing fungal allergy, both prick/puncture and intradermal testing are relevant (50). The reason for allergy to multiple fungi has been postulated to relate to either a common fungal epitope or a genetic predisposition toward fungal allergy in patients with AFRS. Work by Chrzanowski and coworkers (51) identified the presence of an 18-kD protein in allergic mucin, obtained from patients with AFRS, that might represent a fungal panantigen.

Total IgE values are generally increased in patients with AFRS, occasionally to over 1,000 IU/ml (38, 50). Fungus-specific IgG precipitins to the implicated fungus can be demonstrated in most but not all cases (35, 52). However, neither total IgE levels nor fungus-specific IgG precipitins are as strikingly elevated in AFRS as in ABPA, and neither is required to establish the diagnosis of AFRS. A study by Manning and Holman (53) prospectively compared 8 patients with culture-positive *Bipolaris* species AFRS with 10 control subjects with CRS. All eight patients with AFRS had positive skin tests to *Bipolaris* species antigen, as well as positive *Bipolaris* species-specific IgE and IgG. In comparison, 8 of the 10 control subjects had negative results on both skin and serologic testing. Therefore, in the absence of AFRS, allergy to *Bipolaris* is unlikely to be associated with positive *Bipolaris*-specific IgG precipitins.

Histologic Characteristics of Allergic Mucin

The diagnosis of AFRS hinges on confirmation of the presence of allergic mucin in one or more sinus cavities. Allergic mucin is grossly thick, tenacious, and highly viscous. Its color can vary from light tan to brown or dark green (54–56). Histologically, allergic mucin reveals sheets of eosinophils and Charcot–Leyden crystals (26). Fungus must be identified in the allergic mucin either by staining or culture. Traditionally, fungi are identified using silver stains, such as Grocott's or Gomori's methamine silver stain, which stain fungi black or dark brown. Unfortunately, silver-based stains have high specificity but low sensitivity. A more sensitive fluorescein-labeled chitinase stain that stains the chitin layer of the fungal organism (e.g., Fungalase; Anomerics, Baton Rouge, LA) has been described (57). Using this technique, the authors found fungal elements in 54 of 54 unselected CRS cases, whereas fungi were only detected in 41 (76%) of the 54 specimens by using a Grocott stain. Clearly this technique has the potential to improve the diagnostic accuracy of evaluating allergic mucin for the presence of fungi, although it might prove to be overly sensitive for differentiating AFRS from other cases of CRS. The identification of fungi by a fluorescein-labeled chitinase stain is a major tenet of the hypothesis associated with the concept of eosinophilic fungal rhinosinusitis (discussed below).

Otherwise, the sinus mucosa and nasal polyps in AFRS show findings of chronic inflammation, usually with an abundance of eosinophils. Pathologic examination must also be done to confirm the absence of fungal invasion (55).

Pathophysiology

The pathophysiology of allergic fungal rhinosinusitis (AFRS) is most consistent with chronic, intense allergic inflammation directed against colonizing fungi. Histologically, allergic mucin demonstrates intense eosinophilic degranulation, mucostasis, and inspissation (50). A recent study confirmed that fungal antigens from *Alternaria*- and *Cladosporium*-induced Th2 cytokine production (IL-5) *in vitro* by peripheral blood lymphocytes from patients with AFRS compared with healthy control subjects (58).

Pant and colleagues questioned the role of fungal-specific IgE in the pathogenesis of allergic fungal rhinosinusitis (AFRS). Unlike the related condition of classic allergic bronchopulmonary aspergillosis (ABPA), patients with AFRS are indistinguishable from patients with allergic rhinitis and fungal allergy in terms of total serum IgE, levels of *Alternaria alternata*– or *Aspergillus fumigatus*–specific fungal IgE, IgG or IgA levels, or in terms of the percent of fungal specific serum IgE relative to total serum IgE measurements (59). Furthermore, the prevalence of fungal allergy to *Alternaria alternata* or *Aspergillus fumigatus* in AFRS and AFRS-like patients was no different from that observed in allergic rhinitis with fungal allergy (60). This study validly points out that fungal-specific IgE measurements may be a marker of disease in AFRS without contributing importantly to the local tissue pathology. Similar questions

were raised years ago about the importance of *Aspergillus*-specific IgE in the pathogenesis of ABPA (61). This study further serves to emphasize the importance of Th2 cytokines in accounting for eosinophilic inflammation in AFRS. Th2-derived IL-5 and IL-13 are likely involved in the local eosinophil accumulation and subsequent eosinophil-mediated attack of fungal hyphae in mucus.

Diagnostic Dilemmas

None of the diagnostic criteria for AFRS are completely specific to this condition. Fungal allergy is found in about 20% of CRS cases overall. Incidental fungal colonization may occasionally be found in sinus mucus by stain or culture in the absence of allergic mucin. It is therefore the constellation of findings that confirms the diagnosis of AFRS. The biggest diagnostic dilemmas come in cases in which allergic mucin is present but the other criteria for AFRS are not met.

Some cases of CRS have allergic mucin but no detectable fungi in the mucus. These have been labeled "eosinophilic mucin rhinosinusitis" (EMRS) by some authors (62). The sinus CT scan in these cases can also show hyperdensities (42). Although it is possible that some of these cases actually have AFRS, it is noteworthy that they are often completely negative for fungal and other inhalant allergy. This suggests that EMRS is a distinct entity, since most patients with AFRS have other inhalant allergies (63). Many EMRS cases have Samter's triad of nasal polyposis, asthma, and aspirin-induced respiratory disease. It would also be unlikely that AFRS were present if the patient were found to be nonallergic to a fungus cultured from allergic mucin, unless this were considered a contaminant (45). When the patient has allergic mucin and evidence of fungal allergy but no fungi by staining or culture, the patient can be considered to be an "AFRS candidate" (45).

Treatment

Although antifungal medications, including topical amphotericin B and oral terbinafine, have been studied in CRS, there have been no published studies of antifungal medications for treatment of AFRS (*see below*).

POTENTIALLY BROADER ROLE FOR FUNGI IN CHRONIC RHINOSINUSITIS

Few issues in rhinology have engendered as much controversy in the past decade as that of the potential role of fungi as a cause of chronic rhinosinusitis. This can be attributed to the publication by Ponikau and colleagues in 1999 (48). In this paper, the authors demonstrated that viable fungal spores are highly prevalent (near 100% prevalence) in nasal lavage of both patients with CRS and normal control subjects. Multiple fungal species were represented in the nasal lavage, with the prevalence of individual species reflecting the prevalence of spores in regional outdoor air. However, the paper went on to show that fungal hyphae could be identified by stain in the mucus extracted from diseased sinus cavities in 93% of CRS cases. The vast majority of the mucus samples also contained eosinophils and high levels of eosinophil major basic protein (MBP) (64). The authors described the mucus as "allergic mucin" or "eosinophilic mucin" and claimed that most cases of CRS were in fact cases of "eosinophilic fungal rhinosinusitis."

The assertion by Ponikau and coworkers had two major repercussions. First, it shaped much of the research into the pathogenesis of CRS for several years. Second, it blurred the distinction between typical cases of CRS (dubbed "eosinophilic fungal rhinosinusitis") and the small subset of CRS cases classically defined as "allergic fungal rhinosinusitis" (AFRS). Over the ensuing decade, this "fungal hypothesis" of CRS pathogenesis has had its share of supporters (65) and detractors (66). However, with respect to blurring the distinction between "eosinophilic fungal rhinosinusitis" and "allergic fungal rhinosinusitis," most experts have preferred to maintain the distinction between these entities.

What is the Prevalence of Fungal-specific IgE in Allergic Mucin?

In many cases of CRS with allergic mucin, fungal allergy, by skin or *in vitro* testing, is not present. This has led to speculation that local IgE production may underlie the pathogenesis in these cases. Few investigators have actually examined sinus mucus for the presence of fungal-specific IgE levels. In one such study, done by Collins and colleagues (67), 86 patients were classified into AFS; AFS-like; nonallergic fungal eosinophilic rhinosinusitis (NAEFS); and nonallergic, nonfungal eosinophilic sinusitis (NANFES). As expected, all patients with AFS and NAEFS had positive fungal cultures, whereas the AFS-like and NANFES patients had negative cultures. Furthermore, as defined, allergy skin tests were positive in all AFS and AFS-like patients, whereas they were negative in the NAEFS patients. In all, 71% of the allergic mucin samples from AFS patients were positive for fungal IgE to *Alternaria alternata* or *Aspergillus fumigatus*. In contrast, only 16% of the allergic mucin samples from AFS-like sinusitis and only 19% of the allergic mucin samples from NAEFS were positive for fungal IgE. In my opinion, the results of this study point to a difference between allergic and nonallergic patients with eosinophilic mucin. The simultaneous presence of fungal-laden allergic mucin, systemic fungal allergy, and local fungal IgE truly distinguish AFRS from AFS-like and NAEFS. Out of 51 patients with negative serum fungal-specific IgE measurements, only 6 were found to have detectable fungal IgE in the allergic mucin. This suggests that the nearly 90% of cases with negative serum fungal IgE measurements do not have localized fungal IgE-mediated allergy. It is noteworthy that 65% of the patients in this study had fungi identified by culture or fungal staining of the allergic mucin. Therefore, the presence of fungi at some level in allergic mucin was clearly higher than the prevalence of fungal-specific IgE in allergic mucin in this study.

Is There a Special Role for Fungal-specific IgE-independent Eosinophil Accumulation in CRS?

The question of whether fungi can elicit Th2-type eosinophilic responses in the absence of IgE induction has been raised, since many patients with CRS with allergic mucin have no evidence of IgE-mediated allergy to fungi or other allergens ("eosinophilic mucin rhinosinusitis"). As previously mentioned, the study by Collins and coworkers (67) further showed that fungi are often detectable in the allergic mucin in the absence of detectable fungal-specific IgE. It has been shown that superantigens from colonizing *Staphylococcus aureus* induce local IgE production in patients with nasal polyps (68). While this is one possible explanation for local eosinophilic responses, it does not exclude other possibilities. An alternative view is that the predominant allergic mechanism in "nonallergic" patients with CRS is actually a "modified allergic" response to colonizing fungi manifested as a mixed or Th1/Th2 cytokine profile. Although studies of the T lymphocytes within CRS tissue are limited, studies of peripheral blood lymphocytes from patients with CRS found that certain fungi, particularly *Alternaria*, induce IL-5, IL-13, and IFN-γ production with little production of IL-4 (69). No such responses were found in normal subjects.

This profile of cytokines is analogous to what we described in infiltrating T cells in nasal polyps from patients with chronic hyperplastic sinusitis or nasal polyposis (70–72) and what was found by al Ghamdi and coworkers (73) in nonpolypoid allergic versus nonallergic CRS.

The mechanisms responsible for this "modified allergic response" are not yet clear and deserve further study. Analogous paradoxical eosinophilic responses without a clear-cut allergic basis are seen in other conditions, including intrinsic asthma and eosinophilic esophagitis. Other factors may modify classic allergic inflammation in this setting, including influences from bacterial and perhaps fungal colonization and possibly genetic polymorphisms that promote tissue eosinophilia.

The burden of fungal antigen in mucus may be increased by antibiotic use. It can be speculated that this may play a role in fungal sensitization and the intense localized inflammatory response.

Once the modified allergic response is initiated, eosinophil infiltration ensues, with local eosinophil attack of colonizing fungi and buildup of high levels of eosinophil granular proteins in the mucus (64). Mucus stagnation and perhaps epithelial damage by eosinophil basic proteins then contribute to bacterial colonization and/or infection. A viscous cycle of colonization and inflammation is therefore set into motion. In a recent paper, Ponikau and colleagues have demonstrated that fungal hyphae in AFRS mucus tend to "line up" next to eosinophils (64). It is presumed that the eosinophils "attack" the fungal hyphae much as they are known to attack parasitic organisms, resulting in their degranulation (74).

Do Antifungal Medications Have Efficacy for Treatment of CRS?

A double-blind, placebo-controlled trial of topical amphotericin B involving 24 patients treated for 6 months produced a small but statistically significant improvement in sinus mucosal thickening (75). However, a subsequent double-blind, placebo-controlled trial in Europe involving 116 patients treated for 3 months failed to show efficacy over placebo (76). Optimum delivery of a topical antifungal medication to affected sinus areas could certainly be argued as an explanation for failure of antifungal treatment. However, a study of oral terbinafine given at a dose of 625 mg daily versus placebo also failed to show efficacy in terms of symptomatic or radiographic improvement for treatment of CRS in a 12-week randomized controlled clinical trial of 56 patients (77). Therefore, the published clinical trials of antifungal treatment have fallen short of providing compelling proof for the "fungal hypothesis" of CRS pathogenesis. A major limitation of these trials, however, is the lack of demonstration that antifungal treatment actually reduces the burden of colonizing fungi.

CONCLUSIONS

Ambient mold spores are widely distributed in nature, and an estimated 3 to 10% of the world's population is allergic to molds. There are compelling epidemiologic links between mold (fungal) allergy and illnesses such as asthma and asthma with allergic rhinitis. Fungal allergy is more prevalent in areas of high ambient mold spore concentrations. However, epidemiologic studies have failed to demonstrate a direct relationship between fungal allergy and allergic rhinitis. Studies of indoor dampness or mold exposure in relation to rhinitis symptoms have also failed to demonstrate a strong relationship, although a relationship was demonstrated between high in-home fungal concentrations and development of allergic rhinitis in the first 5 years of life in one birth cohort.

Fungal allergy is clearly linked to a subset of chronic rhinosinusitis known as allergic fungal rhinosinusitis (AFRS). This condition represents an intense allergic response against colonizing fungi giving rise to formation of allergic (eosinophilic) mucin, mucostasis, and sinus opacification. Fungal allergy is universally found in this condition. On the other hand, a broader role for colonizing fungi has been postulated in CRS owing to the demonstration of fungi associated with eosinophil-containing mucus of the vast majority of cases of CRS, and *in vitro* studies demonstrating that certain fungi, particularly *Alternaria*, elicit a "modified" allergic response, independent of IgE, in patients with CRS but not in healthy control subjects. Although published trials of antifungal treatment have fallen short of providing compelling proof for this "fungal hypothesis" of CRS pathogenesis, further studies are clearly warranted in this area.

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